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Neuropeptides 39 (2005) 559–567
Neuropeptides
www.elsevier.com/locate/npep

Localization of opioid receptor antagonist [^{3}H]-LY255582 binding sites in mouse brain: Comparison with the distribution of mu, delta and kappa binding sites

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Received 25 August 2005; accepted 24 September 2005
Available online 9 November 2005

Abstract

Agonist stimulation of opioid receptors increases feeding in rodents, while opioid antagonists inhibit food intake. The pan-opioid antagonist, LY255582, produces a sustained reduction in food intake and body weight in rodent models of obesity. However, the specific receptor subtype(s) responsible for this activity is unknown. To better characterize the pharmacology of LY255582, we examined the binding of a radiolabeled version of the molecule, [^{3}H]-LY255582, in mouse brain using autoradiography. In mouse brain homogenates, the K_d and B_{max} for [^{3}H]-LY255582 were 0.156 ± 0.07 nM and 249 ± 14 fmol/mg protein, respectively. [^{3}H]-LY255582 bound to slide mounted sections of mouse brain with high affinity and low non-specific binding. High levels of binding were seen in areas consistent with the known localization of opioid receptors. These areas included the caudate putamen, nucleus accumbens, claustrum, medial habenula, dorsal endopiriform nucleus, basolateral nucleus of the amygdala, hypothalamus, thalamus and ventral tegmental area. We compared the binding distribution of [^{3}H]-LY255582 to the opioid receptor antagonist radioligands [^{3}H]-naloxone (mu), [^{3}H]-naltrindole (delta) and [^{3}H]-norBNI (kappa). The overall distribution of [^{3}H]-LY255582 binding sites was similar to that of the other ligands. No specific [^{3}H]-LY255582 binding was noted in sections of mu-, delta- and kappa-receptor combinatorial knockout mice. Therefore, it is likely that LY255582 produces its effects on feeding and body weight gain through a combination of mu-, delta- and kappa-receptor activity.

Keywords: Autoradiography; Inverse agonist; Feeding behavior; Neuroanatomy; Obesity

Abbreviations: AcbC, nucleus accumbens,core; BLA, basolateral nucleus of the amygdala; CA3, CA3 region of the hippocampus; cl, claustrum; CG, central gray; CI, 977, (−)-*N*-methyl-*N*-[7-(1-pyrrolidinyl)-1-oxaspiro[4,5]dec-8-yl]-4-benzofuranacetamide; CPu, caudate; CTR, central thalamic nuclei; DAMGO, [D-Ala^2,MePhe^4,Gly-ol^5] enkephalin; Den, dorsal endopiriform; DPDPE, cyclic [D-penicillamine^2,D-penicillamine^5] enkephalin; DSLET, [D-Ser^2,Leu^5] enkephalin-Thr; fr, fasciculus retroflexus; LH, lateral hypothalamus; MHb, medial habenula; Nor-BNI, nor-binaltrophimine; PN, paranigral nucleus; PVN, paraventricular nucleus of the hypothalamus; RMC, red nucleus,magnocellular; SA, specific activity; SKF 10047, (+)-*N*-allylnormetazocine hydrochloride; SuG, superficial gray layer of the superior colliculus; Triple KO, delta (−/−),mu (−/−) and kappa (−/−) genes removed from mouse strain; U69,593, (+)-(5α,7α,8β)-*N*-methyl-*N*-[7(1-pyrrolidinyl)-1-oxaspiro[4,5]dec-8-yl]-benzeneacetamide; 3-PPP, 3-(3-hydroxyphenyl)-*N*-(1-propyl)piperidine; 4-PP, 3-4-dimethyl-4-(3-hydroxyphenyl)piperidine.

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doi:10.1016/j.npep.2005.09.006

1. Introduction

Opioid peptides are known to modulate numerous physiological responses both within the central nervous system and in the periphery. Opioids regulate antinociception, motivation and reward, addiction, gastrointestinal motility, appetite, blood pressure, neuroendocrine hormone secretion, locomotion, thermoregulation, stress responses and the processing of sensory information (Mansour et al., 1987; Kieffer, 1995; Reisine, 1995; Vaccarino and Kastin, 2001). The brain areas associated with these responses contain one or more of the opioid receptor subtypes, mu, delta and kappa. Pharmacologically, delta- and mu-receptors are characterized by high affinity for the endogenous opioids β-endorphin and the enkephalins. Selective ligands for the delta-receptor include the peptide agonist cyclic [D-penicillamine^2, D-penicillamine^5] enkephalin (DPDPE) and the antagonist naltrindole. The mu-receptor exhibits selectivity for the agonists DAMGO and morphine, and the antagonist, naloxonazine. The dynorphins appear to be endogenous agonists for the kappa-receptor, which has high affinity for the agonists (−)-*N*-methyl-*N*-[7-(1-pyrrolidinyl)-1-oxaspiro[4,5]dec-8-yl]-4-benzofuranacetamide (CI, 977) and (+)-(5α,7α,8β)-*N*-methyl-*N*-[7(1-pyrrolidinyl)-1-oxaspiro[4,5]dec-8-yl]-benzeneacetamide (U69, 593) and the antagonist nor-binaltrophimine (nor-BNI) (Kieffer, 1995; Reisine, 1995).

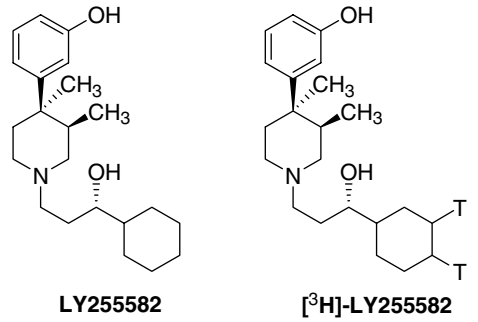
Mu-, delta- and kappa-opioid receptors each exhibit a distinctive neuroanatomical distribution that provides insight to their physiological role. Studies using agonist radioligands have characterized the distribution of opioid receptor subtypes in sections of mouse brain. Using [^{3}H]-DAMGO, a high level of mu-opioid receptor binding occurs in the caudate putamen (patches), nucleus accumbens, endopiriform nucleus, amygdaloid nuclei, superficial gray layer of the superior colliculus, red nucleus and the interpuduncular nucleus (Kitchen et al., 1997). Delta-receptor binding sites (defined with [^{3}H]-deltorphin I) have high density within the olfactory bulb, olfactory tubercle, caudate putamen (diffuse), basolateral amygdala and areas of the cortex (Goody et al., 2002). Interestingly, [^{3}H]-CI-977 binding to kappa sites in the mouse brain is low compared to mu and delta sites with the highest kappa binding occurring in the nucleus accumbens, claustrum, olfactory tubercle, ventral pallidum, deep layers of the cortex, hypothalamus and periaqueductal gray (Slowe et al., 1999).

LY255582, (3*R*,4*R*)-3,4-dimethyl-1-[(3*S*)-3-hydroxy-3-cyclohexyl-propyl]-4-(3-hydroxyphenyl)piperidine, has high affinity for mu- (0.41 nM), delta- (5.2 nM), and kappa-receptors (2 nM) in rat brain (mu and delta) and guinea pig cortex homogenates (kappa), respectively (Mitch et al., 1993). Emmerson et al. (2004) reported sodium dependent increases in receptor binding affinity and inverse agonist activity of LY255582 in membranes expressing the cloned human mu-, delta-, or kappa-receptors. LY255582 potently decreases food intake and body weight in rodents (Levine et al., 1991; Shaw et al., 1991; Shaw, 1993; Statnick et al., 2003b). Unlike the classic opioid antagonist naltrexone, LY2555582 produced a sustained weight loss in obese Zucker rats lasting 70 days without signs of tolerance (Shaw et al., 1991; Shaw, 1993). To better understand the mechanism by which LY255582 reduces food intake and body weight, we synthesized [^{3}H]-LY255582 and evaluated its binding distribution in mouse brain, and compared it to the distribution of the opioid receptor antagonist radioligands [^{3}H]-naloxone, [^{3}H]-naltrindole and [^{3}H]-norBNI. Studies were conducted in sections of mouse brain due to the availability of opioid receptor knockout strains and the fact that mouse models of obesity and feeding behavior are common.

2. Materials and methods

2.1. Synthesis of [^{3}H]-LY255582

The precursor for tritiation, 3-[1-(3-cyclohex-3-enyl-3(*S*)-hydroxy-propyl)-3(*R*), 4(*R*)-dimethylpiperidin-4-yl]-phenol, was prepared according to a previously reported synthetic route (Statnick et al., 2003a). The desired enantiomers of 3-[1-(3-cyclohex-3-enyl-3-hydroxy-propyl)-3,4-dimethyl-piperidin-4-yl]-phenol were separated by flash chromatography on silica gel eluting with 0.5–10% (10% ammonium hydroxide in ethanol) in chloroform. Tritiation of 3-[1-(3-cyclohex-3-enyl-3(*S*)-hydroxy-propyl)-3(*R*),4(*R*)-dimethylpiperidin-4-yl]-phenol was performed according to previously reported methods (Statnick et al., 2003a). The specific activity of [^{3}H]-LY255582 (as determined by mass spectrometry) was 60 Ci/mmol. The structures of LY255582 and [^{3}H]-LY255582 can be seen in Fig. 1.



LY255582 **[^{3}H]-LY255582**

Fig. 1. Chemical structures of LY255582 and [^{3}H]-LY255582.